DISTURBANCE OF NUCLEIC ACID METABOLISM PRODUCED BY THERAPEUTIC DOSES OF X AND GAMMA RADIATIONS. PART II: ACCUMULATION OF PENTOSE NUCLEOTIDES IN CYTOPLASM AFTER IRRADIATION.

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Increase in Cytoplasmic Absorption at 2537 Å.

A striking increase in the ultraviolet absorption of the cytoplasm of cells after irradiation has been found in 11 out of 15 pairs of biopsy specimens from gamma-ray treated cases, and in 13 out of 17 pairs of biopsy specimens from

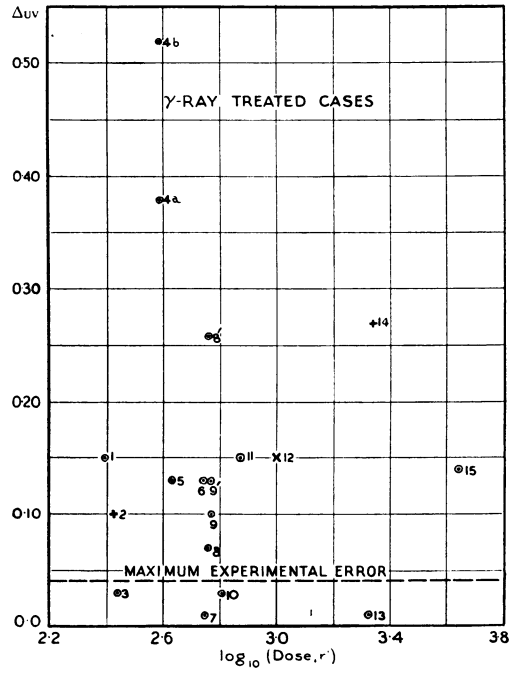

FIG. 1.—Measurements on gamma-ray treated cases. Graph showing dependence of increase in ultraviolet absorption of cytoplasm (Δ_{UV}) upon *dose*. (The numbers refer to the case numbers in the series.)

\+ Biopsy 0 min. after end of irradiation.
○ ,, 80 ,, ,, ,, ,,
× ,, 9 hr. ,, ,, ,,
— — — — Maximum experimental error.

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cases treated with X radiation. The histology of the tissues examined is given in Table I, and the quantitative experimental results are summarized in Figs. 1, 2, 3, 4, and 5.*

The order of magnitude of the observed increase in the optical density of the cytoplasm for absorption of ultraviolet radiation of wave-length 2537 Å is 0·15 for sections of approximate thickness 2μ. The interpretation of this will be discussed below, but it may be noted here that this increase in density

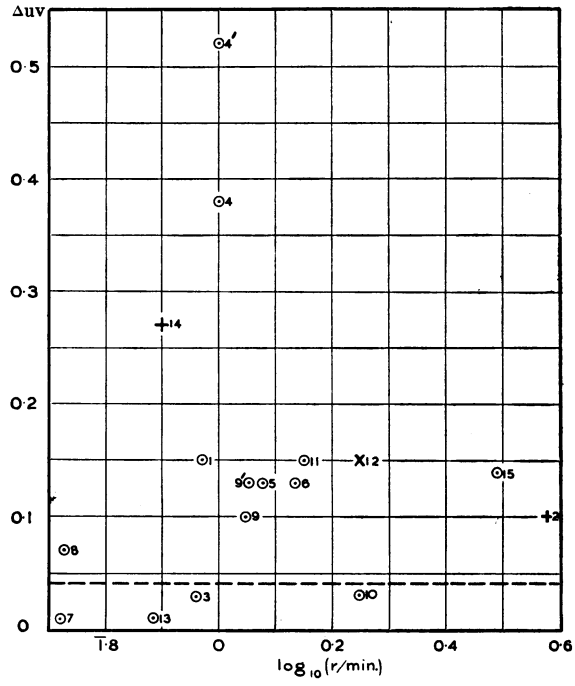

FIG. 2.—Measurements of gamma-ray treated cases. Graph showing dependence of increase in ultraviolet absorption of cytoplasm (Δ_{uv}) upon dose rate. (The numbers refer to the case numbers in the series.)

\+ Biopsy 0 min. after end of irradiation
⊙ ,, 80 ,, ,, ,, ,,
× ,, 9 hr. ,, ,, ,,
— — — — Maximum experimental error.

corresponds to a local concentration in the cytoplasm of the affected cells of 3·5 per cent. of phyto-nucleic acid or the corresponding nucleotides; the absorbing material is not thymonucleic acid as shown by the negative Feulgen and Dische reactions (cf. Dische, 1930; Lison, 1936).

The increase in cytoplasmic absorption after irradiation is shown typically by proliferating and differentiating cells; with fully differentiated cells there may be a small change,† but typically there is no change. As yet no quantitatively significant differences in behaviour have been observed between the

* The author will be pleased to supply these results in tabular form to anyone interested.

† The possibility of diffusion of absorbing substances of low molecular weight introduces uncertainty with regard to the histological localization of small changes.

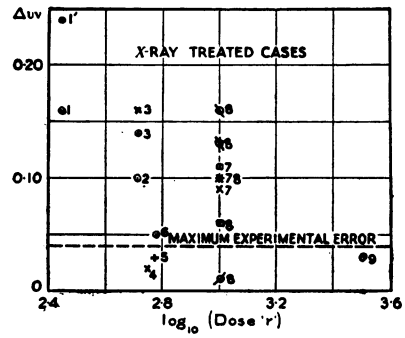

FIG. 3.

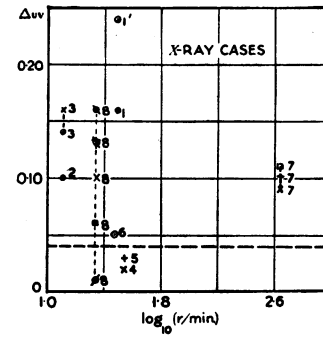

FIG. 4.

FIG. 3.—Measurements of X-ray treated cases. Graph showing dependence of increase in ultraviolet absorption of cytoplasm (Δ_{uv}) upon *dose*. (The numbers refer to the case numbers in the series.)

⊙ Biopsy 80 min. after end of irradiation.
\+ ,, 18 hr. ,, ,, ,,
× ,, 24 ,, ,, ,, ,,
⊙ ,, 7 days ,, ,, ,,
† ,, 14 ,, ,, ,, ,,
⊙ ,, 30 ,, ,, ,, ,,
⊡ ,, 56 ,, ,, ,, ,,
— — — — Maximum experimental error.

FIG. 4.—Measurements on X-ray treated cases. Graph showing dependence of increase in ultraviolet absorption of cytoplasm (Δ_{uv}) upon dose rate. (The numbers refer to the case numbers in the series.)

⊙ Biopsy 80 min. after end of irradiation.
\+ ,, 18 hr. ,, ,, ,,
× ,, 24 ,, ,, ,, ,,
⊙ ,, 7 days ,, ,, ,,
† ,, 14 ,, ,, ,, ,,
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⊡ ,, 56 ,, ,, ,, ,,
— — — — Maximum experimental error.

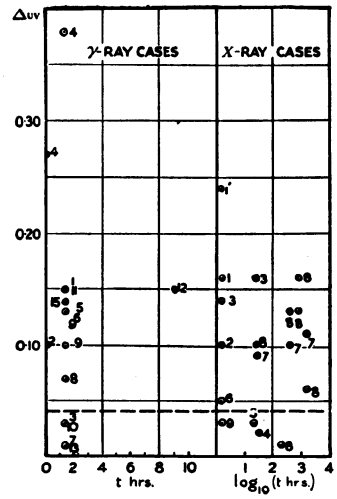

FIG. 5.—Graphs showing dependence of increase in ultraviolet absorption of cytoplasm (Δ_{uv}) upon time of biopsy after end of irradiation. (The numbers refer to the case numbers in the series.)

t hr. : Time of biopsy after end of irradiation.

TABLE I.—*Gamma-ray Treated Cases.*

Case No.	Histology of structures examined.
1	Hyperplastic keratinizing squamous epithelium adjacent to keratinizing squamous-cell carcinoma of lower lip.
2	Normal skin of tadpole.
3	Hyperplastic keratinizing squamous epithelium adjacent to squamous-cell carcinoma of lower lip.
4	Hyperplastic keratinizing squamous epithelium continuous with squamous-cell carcinoma of lower lip.
5	Hyperplastic keratinizing squamous epithelium continuous with squamous-cell carcinoma of ear.
6	Keratinizing squamous-cell carcinoma of lower lip.
7	Hyperplastic keratinizing squamous epithelium continuous with squamous-cell carcinoma of scalp.
8	Hyperplastic keratinizing squamous epithelium adjacent to basal-cell carcinoma of scalp.
9	Hyperplastic keratinizing squamous epithelium adjacent to basal-cell carcinoma of skin of temporal region.
10	Hyperplastic keratinizing squamous epithelium continuous with keratinizing squamous-cell carcinoma of face.
11	Basal-cell carcinoma of skin of face.
12	Anaplastic squamous-cell carcinoma of cervix.
13	Keratinizing squamous-cell carcinoma of lower lip.
14	Hyperplastic keratinizing squamous epithelium adjacent to basal-cell carcinoma of skin of outer canthus.
15	Squamous-cell carcinoma of cervix.

X-ray Treated Cases.

1	Hyperplastic keratinizing squamous epithelium adjacent to squamous-cell carcinoma of skin of temporal region.
2	Squamous-cell carcinoma of fauces.
3	*a*. Normal squamous epithelium of hard palate adjacent to— *b*. Large spindle-cell sarcoma of antrum.
4	Keratinizing squamous-cell carcinoma of lower lip.
5	Hyperplastic keratinizing squamous epithelium adjacent to squamous-cell carcinoma of lower lip.
6	Hyperplastic keratinizing squamous epithelium adjacent to anaplastic carcinoma of skin of thigh.
7	Squamous-cell carcinoma of vulva.
8	Keratinizing squamous carcinoma of vulva.
9	Squamous-cell carcinoma of fauces.

cells of the malignant tumours examined—mostly squamous and basal-cell carcinomata and one large spindle-cell sarcoma—and the homologous cells of the adjacent normal and hyperplastic tissues. Further, a similar increase in ultraviolet absorption has been observed with living cells in culture (chick heart and choroid fibroblasts and skin epithelial cells) irradiated in situ on the microscope stage by means of a radium applicator placed on the objective, giving a dose of 340 r of gamma radiation in one hour.*

Typical changes with normal squamous epithelium are shown in Figs. 6 and 7 (Case 3a). These sections show a striking increase in ultraviolet absorption, $\Delta_{uv} = 0{\cdot}14$, 80 min. after 516 r X-radiation in 40 min. (Fig. 6).

* These experiments have been made in conjunction with Dr. A. W. Calm and Dr. I. Glücksmann, and if possible will be described later.

These photographs show clearly the great increase in cytoplasmic absorption in the growing cells of the stratum basale and the differentiating cells of the stratum spinosum. The microphotographs at magnification × 670 and the microphotometer tracings show that the cytoplasmic absorption increase is very much greater in the incompletely differentiated cells than in those almost fully differentiated. Further, the microphotometer tracing shows that the concentration of absorbing substances accumulating after irradiation is greatest immediately outside the nuclear membrane and decreases toward the periphery of the cytoplasm (cf. Caspersson, 1940, fig. 5). Fig. 7, Case 3a, shows comparable biopsy specimens from the same case before and 24 hr. after 516 r in 40 min. Measurements show that in the areas of more completely differentiated cells the absorption increase persists without significant change, but that there is very considerable recovery in the more undifferentiated cells.

The changes shown by *hyperplastic epithelium* adjacent to squamous-cell carcinomata are shown in Figs. 8–11 (Case 1) and by the hyperplastic epithelium adjacent to basal-cell carcinomata of the skin in Fig. 14 (Case 8). (All these cases were treated with gamma radiation.) In all instances there is an obvious and quantitatively significant increase in the ultraviolet absorption of the cytoplasm.

The changes shown by *squamous-cell carcinomata* are exemplified by Figs. 12 and 13 (Case 8) (X-ray treatments).

Case 12 provided sections from a recurrent anaplastic squamous-cell carcinoma of the cervix before and 9 hr. after 1000 r of gamma radiation given in 9·47 hr. by means of a specially constructed applicator. The measurements made on the comparable areas of tumour tissue show that $\Delta_{uv} = 0{\cdot}15$, and

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DESCRIPTION OF PLATES.

FIGS. 6 and 7.—Case 3a. Normal squamous epithelium of hard palate. × 300.
6. Sections: Control and 80 min. after 516 r in 40 min. ($\Delta_{uv} = 0{\cdot}14$).
7. Sections: Control and 24 hr. after 516 r in 40 min. ($\Delta_{uv} = 0{\cdot}16$).

FIGS. 8–11.—Case 1. Hyperplastic epithelium adjacent to squamous-cell carcinoma of lower lip, treated with double radium mould. × 300. Sections: Control and 80 min. after 248 r in 4·45 hr. The observed increase in cytoplasmic absorption at 2537 Å ($\Delta_{uv} = 0{\cdot}15$) corresponds to that of a 3·5 per cent. solution of phytonucleic acid or its constituent ribo-nucleotides.
8. Unmarked plate.
9. Plate marked for measurement with non-recording microphotometer.
10. Plate marked for measurement with recording microphotometer.
11. Disappearance of cytoplasmic absorption after acid hydrolysis and extraction with absolute alcohol.

FIGS. 12 and 13.—Case 8. Keratinizing squamous carcinoma of vulva. × 300.
12. Sections: Control and 14 days after 1000 r at 22 r/min. ($\Delta_{uv} = 0{\cdot}13$).
13. Sections: Control and 30 days after 1000 r at 22 r/min.

FIG. 14.—Case 8. Hyperplastic epithelium adjacent to basal-cell carcinoma of scalp. × 300. Sections: Control and 80 min. after 575 r in 18·0 hr. ($\Delta_{uv} = 0{\cdot}07$—a significant increase). Measurements on marked plate show no change in the nucleic acid content of the nuclei.

FIG. 15.—Case 3b. Large spindle-cell sarcoma of antrum. × 300. Sections: Control and 80 min. after 516 r in 40 min.

FIGS. 16 and 17.—Photomicrographs showing increase in fluorescence after gamma irradiation. Case 1. Squamous-cell carcinoma of lip. Sections: Control and 80 min. after 248 r in 4·45 hr.
16. Fluorescence with exciting radiation of wave-length 2537 Å.
17. „ „ „ „ 2950–4250 Å.

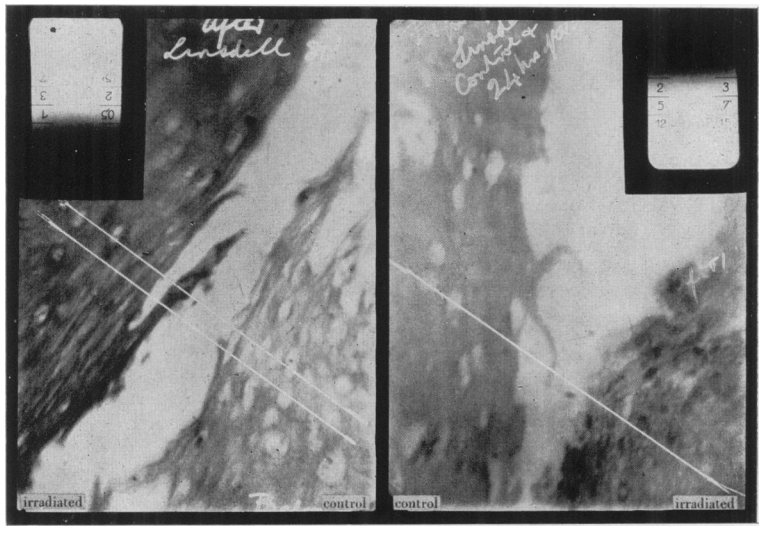
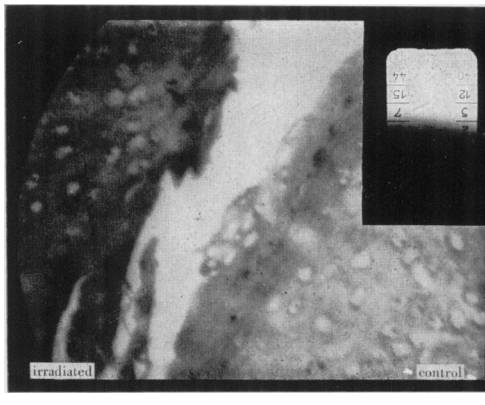


FIG. 6.

FIG. 7.



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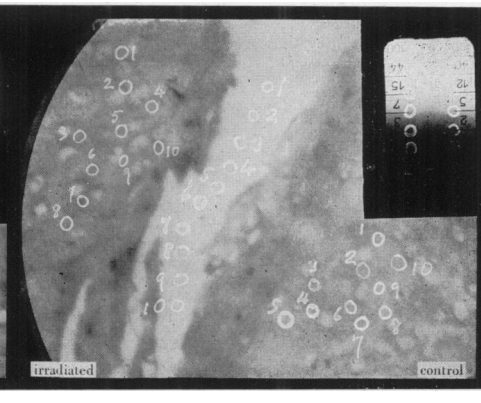

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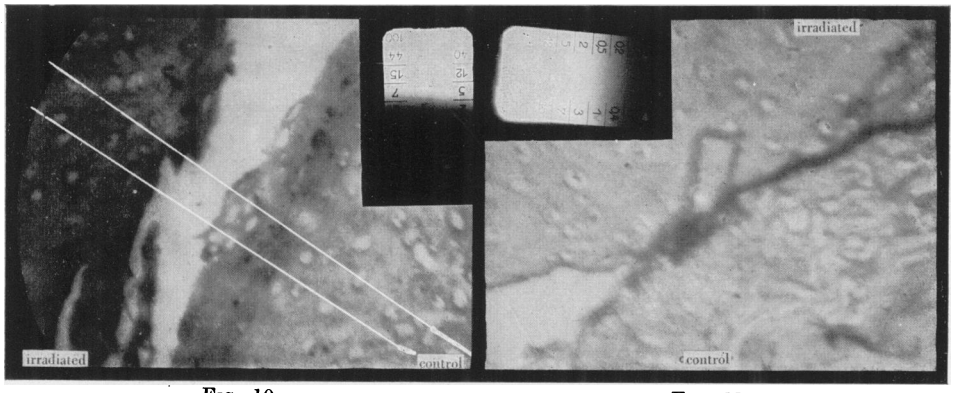

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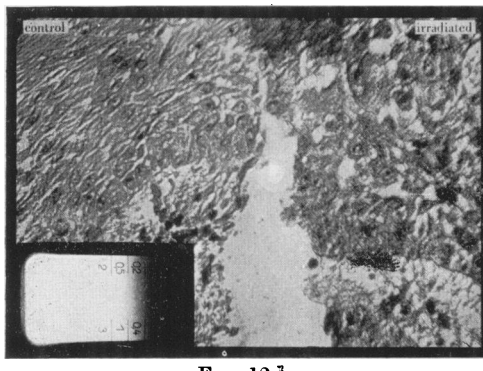

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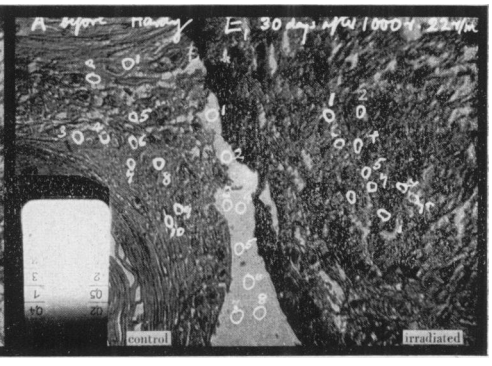

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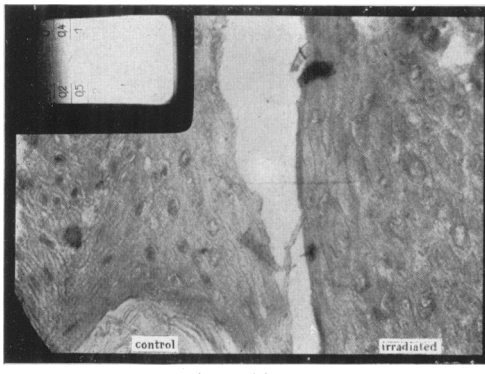

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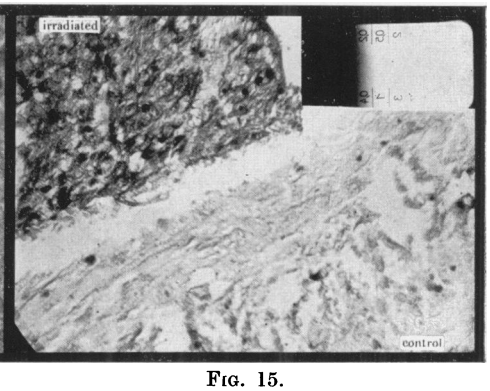

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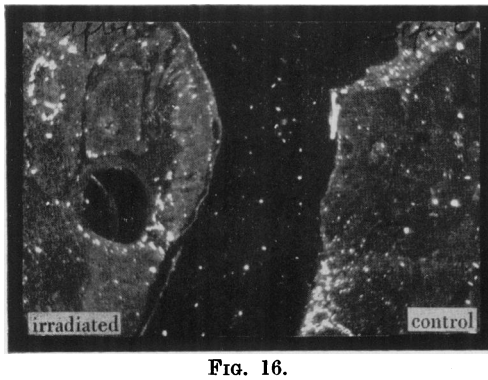

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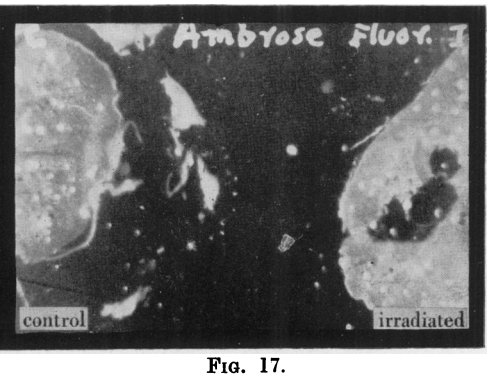

FIG. 17.

it is evident that recovery has been far from complete during the 9-hr. interval following irradiation. Examples of the changes observed after X-ray treatment of carcinoma of the vulva are shown in Figs. 12, 13 (Case 8), and similar measurements were also made with Case 7. In both these cases the clinical response was unsatisfactory, and it is of interest that in Case 7 the cytoplasmic absorption increase persists without significant change 56 days after 1000 r X-radiation given at 444 r/min. Similarly with Case 8 the absorption increase persists for 30 days, but there is evidence of recovery after 56 days.

Microphotographs of a large spindle-cell *sarcoma* of the antrum are shown in Fig. 15 (Case 3b), and demonstrate a very striking increase of cytoplasmic absorption; $\Delta_{uv} = 0{\cdot}26$ in the tumour cells 80 min. after 516 r given in 40 min.

The dependence of the increase in ultraviolet absorption of the cytoplasm (Δ_{uv}) upon the dose is shown for the gamma-treated cases in Fig. 1 and for the X-ray-treated cases in Fig. 3. The increase in ultraviolet absorption does not increase with the dose of radiation, being approximately constant for single doses ranging from 250–4400 r units of gamma radiation and 280–1000 r units of X-radiation. It is evident that the " saturation " values for Δ_{uv} are already reached at the smallest doses examined. The dose rate appears to be a factor of importance—see Figs. 2 and 4—the increase in cytoplasmic absorption showing approximately constant values for dose rates 1·00–444 r/min., but low values at dose rates less than 0·9 r/min. with gamma radiation. In this discussion the great experimental error renders unnecessary the introduction of the wave-length effect factor (cf. Mitchell, 1940). In Fig. 5, giving the dependence of Δ_{uv} upon the time of the biopsy after the end of irradiation, is shown the great variation in the rate of recovery processes ; in typical radiosensitive cases approximately complete recovery occurs after 24 hours. It is of interest to note that the cytoplasmic absorption increase can persist unchanged even for as long as 56 days, and it is tempting to suggest that this failure of metabolic recovery may perhaps be correlated with clinical radioresistance.

Ultraviolet Absorption Spectrum of Accumulating Substances.

Fig. 18 curve (1) shows the *approximate* spectrum of the increase in optical density of the sections of a large spindle-cell sarcoma of the antrum at 80 min. after 516 r X-radiation (Case 3). The sections were of nominal thickness 6μ, and the measured difference in thickness of the control and irradiated sections was less than 0·3μ. The measurements in Fig. 18 were made by the spectroscopic image method and refer to selected small homogeneous areas of the tumour.

Fig. 19 shows the " difference absorption spectrum " for Cases 1 and 4 (gamma ray series) measured by the macroscopic method, using the Hilger Spekker photometer with sections 48μ in thickness.

The form and position of the short wave-length absorption band near 2600 Å correspond to the adenine chromophor (cf. Holiday, 1930 ; Myrbäck, Euler and Hellström, 1932; Heyroth and Loofbourow, 1934*). (The presence

* Also unpublished measurements by Dr. C. B. Allsopp on yeast adenylic acid and by myself on muscle adenylic acid.

of uracil or of small amounts of guanine cannot be excluded.) At the wave-length 2537 Å the observed values of $\Delta_{uv} = 0{\cdot}89$ for Case 3 (X-ray series) corresponds to a concentration of yeast adenylic acid, 5·6 per cent. for a layer 6μ in thickness. This value is exceptionally high; e.g. Case 1 (gamma ray

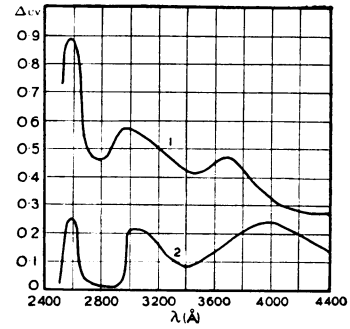

FIG. 18.—Approximate absorption spectrum of substances accumulating after X-irradiation. Case 3. Large spindle-cell sarcoma of antrum. Sections: Control and 80 min. after 516 r in 40 min. (Nominal thickness 6μ.)
Curve 1. Absorption spectrum of accumulating substances.
,, 2. Absorption spectrum of accumulating substances after exposure of fixed sections to ultraviolet radiation of wave-length 2537 Å for 575 hr.

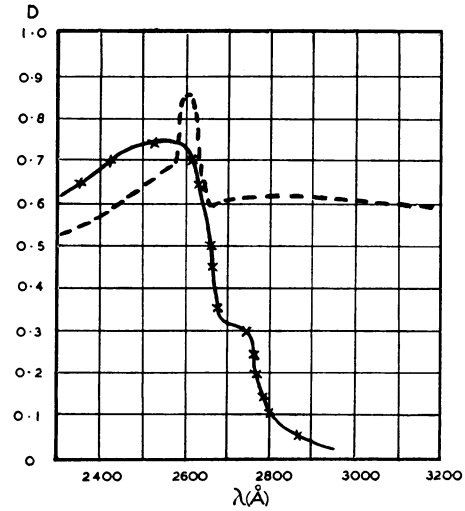

FIG. 19.—Difference absorption spectrum of control and irradiated sections measured by macroscopic method. (Sections 48μ thickness.)
— — — — Case 1. Control and 80 min. after 248 r gamma radiation in 4·45 hr.
×———× Case 4. Control and 80 min. after 388 r gamma radiation in 6·42 hr.

series) gave a value 1·8 per cent. The chromophoric group responsible for the absorption at longer wave-lengths has not yet been identified.

It is evident from Figs. 18 and 19 that the absorption spectrum of the substances accumulating after X and gamma irradiation may differ significantly in some cases from that described by Loofbourow, Dwyer and Lane

(1940) as characteristic of the proliferation promoting " inter-cellular hormones " produced by ultraviolet irradiation of living cells.

It is probable that the two absorption bands with maxima at approximately 3020 Å and 3670 Å are due to a different chromophoric group from that responsible for the band at 2580 Å, as shown by selective photochemical decomposition (Fig. 18, curve 2).

Fluorescence of Irradiated Tissues.

A small increase in fluorescence after X and gamma irradiation has been found, corresponding to the increase in ultraviolet absorption. The observed bluish-white fluorescence is of low intensity, but fluorescence microphotographs

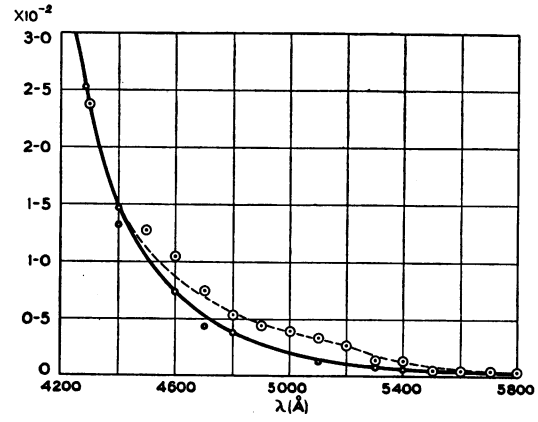

FIG. 20.—Case 1. Squamous-cell carcinoma of lip.
.—.—.—. Approximate spectral distribution of fluorescent radiation due to substances accumulating in tissue after gamma irradiation. Sections: Control and 80 min. after 248 r in 4.45 hr.
○—○—○ Approximate fluorescent spectrum of dried nucleoprotein layer. Wave-length of exciting radiation: 2950–4250 Å.

comparing the control and irradiated tissues have been obtained in a number of cases (1 and 4 of the gamma ray series; 2 and 3 of the X-ray series). Figs. 16 and 17 (Case 1) are typical. No fluorescence was observed after the interposition of a layer of glass plates which excludes wave-lengths shorter than 3300 Å. Experiments with fresh unfixed biopsy specimens showed an apparently similar fluorescence which was not affected by fixation by Susa-alcohol or by heat. The microphotographs show that in epithelial structures the fluorescence is greatest in the basal-cell layer and adjacent parts of the stratum spinosum; the keratinizing structures produce considerable scattering of radiation. The fluorescence does not differ significantly in intensity in the adjacent normal, hyperplastic and neoplastic squamous epithelial structures.

Quantitative measurement of the fluorescence spectrum of the substances accumulating after irradiation is difficult on account of the low intensity. An approximate method employing photographic photometry has been used, and in Fig. 20 is shown the spectral distribution of the fluorescent radiation due to the substances accumulating in a squamous-cell carcinoma of the lip

(Case 1) 80 min. after 248 r gamma radiation in 4·45 hr. ; in the same figure is included the approximate fluorescence spectrum of a dried layer of thymus nucleo-protein. In these experiments the wave-length of the exciting radiation was 2950–4250 Å.

Histochemical Evidence for the Presence of Pentose in the Cytoplasm after Irradiation.

The reaction with aniline acetate and concentrated hydrochloric acid applied as a histochemical test for pentoses is almost invariably positive in the cytoplasm of irradiated cells showing increased ultraviolet absorption at 2537 Å. Bial's orcinol reaction has been found to be less sensitive, but it is positive in specimens showing strongly positive aniline acetate reactions. The colours given by both the aniline acetate and orcinol reactions were transient and only persisted for several minutes. Irradiated sections showing increase in ultraviolet absorption also showed positive colour reactions with alpha-naphthol and concentrated sulphuric acid, and with phloroglucinol and concentrated hydrochloric acid, but with these reactions the sections were not satisfactory for microscopical examination. In normal, hyperplastic and neoplastic keratinizing squamous epithelial structures after X and gamma irradiation the positive pentose reactions are given by the cytoplasm of the basal cells and of many of the spinous cells, but with much less intensity by the cells of the stratum spinosum immediately beneath the stratum granulosum. In a basal-cell carcinoma of the skin (Case 8 of the X-ray series) there was only a slight increase after irradiation in the faint pentose reactions given by the cytoplasm of the tumour cells. The most strongly positive pentose reactions observed after irradiation were given by the cytoplasm of the cells of a large spindle-cell sarcoma (Case 3 of the X-ray series).

In *unirradiated* epithelial structures—normal, hyperplastic and neoplastic —faintly positive pentose reactions are often observed in the cytoplasm of cells of the basal layer and of the adjacent parts of the stratum spinosum. This result is in agreement with the findings of Caspersson (1941) and Caspersson and Schultze (1938, 1939). In both the control and irradiated sections pentose reactions are shown by a large number of the nucleoli ; as a rule there is no other nuclear staining, in agreement with the well-known failure of desoxyribose to give strong microchemical reactions for pentoses.

It is important to note that in no instance was a positive Feulgen reaction shown by cytoplasmic structures in control or irradiated tissues, so that the presence of desoxypentoses in the cytoplasm can be excluded.

Histochemical Evidence for the Presence of Purine and Pyrimidine Derivatives in the Cytoplasm after Irradiation.

The diazo reaction with diazotized sulphanilic acid (cf. Clara, 1934) failed to show any convincing difference between the control and irradiated sections. It was evident that the tyrosine and histidine residues of the proteins gave intense colour reactions which might mask any difference due to substances accumulating after irradiation. To avoid this difficulty, the sections were benzoylated before diazotization. The Schotten-Baumann reaction with

tyrosine is, of course, well known, and also it has long been recognized (cf. Kossel and Edlbacher, 1915; Ashley and Harington 1930) that attempted complete benzoylation of histidine and histamine leads to breaking of the iminazol ring. The diazo derivatives of many purine and pyrimidine compounds are well known (cf. Burian, 1901, 1904; Steudel, 1906; H. Fischer, 1909), but the diazo reactions of the corresponding benzoyl derivatives do not appear to have been described. Subsidiary experiments showed that after benzoylation adenine and uracil give orange-yellow colours, while guanine gives an intense crimson colour reaction, with diazotized sulphanilic acid followed by the addition of alkali.

The benzoylation of the sections by means of benzoyl chloride in the presence of aqueous NaOH requires great care and is best carried out at room temperature.

Method.

The control and irradiated sections are mounted side by side on a glass slide. After removal of the paraffin wax, a few drops of an approximately 2N aqueous NaOH solution are added and then a few drops of benzoyl chloride. A covers lip is placed on the sections, which are left at room temperature for 1–6 or even 12 hours. At the end of this time concentrated HCl is run underneath the cover slip, which floats off. The slide is washed with distilled water, then with 80 per cent. alcohol and finally with absolute alcohol.

The diazotization is carried out using Ehrlich's diazo reagent-diazotized sulphanilic acid (cf. e.g. Hutchison and Hunter, 1935, p. 250). Gentle warming is advisable. The diazo reagent is poured off and one spot of 2N NaOH added. Then, after several minutes the sections are washed in 80 per cent. alcohol, absolute alcohol and xylol and mounted in canada balsam.

In a number of the irradiated specimens showing increased ultraviolet absorption and positive pentose reactions, the diazo reaction after benzoylation showed a quite definite brownish-red staining of the cytoplasm of the basal and many of the spinous cells in normal and hyperplastic epithelium and in squamous-cell carcinomata. Only a faint yellow colour was shown by the epithelial structures on the control side, where in some cases the reaction was greatest in the basal cells. In the case of the specimens of the large spindle-cell sarcoma examined (Case 3), on the control side the tumour cells showed only a faint yellow cytoplasmic staining, but after irradiation there was usually a brownish-red cytoplasmic staining of the tumour cells. In no instance was there intense nuclear staining in either the control or irradiated sections, although many of the nucleoli in both the sections show brown staining. Thus, the diazo reactions after benzoylation are consistent with the presence in the cytoplasm of the irradiated cells of adenine, small amounts of guanine and perhaps also uracil.

As supplementary reactions, Hunter's diazo reaction (Hunter, 1936) in the presence of hydroxylamine and Na_2CO_3 invariably gave yellow but never red colour reactions; this finding makes the presence of thymine unlikely.

The histochemical application of the murexide reaction is notoriously difficult; negative murexide reactions were invariably given by both the control and irradiated sections. This finding would, of course, be consistent

with the presence of adenine, and also of alloxazine (Kuhn and Bär, 1934), but a negative murexide reaction cannot be regarded as very significant. Weidel's reaction (cf. Strauss and Koulén, 1929; Winterstein and Somló, 1933) is invariably negative; also Wheeler and Johnson's reaction is negative.

The presence of a positive diazo reaction after benzoylation in the cytoplasm of irradiated cells runs parallel with the finding of positive pentose reactions. The specimens from the following cases have been examined: Nos. 1, 4, 13, 15 of gamma ray series and 2, 3 of X-ray series.

Interpretation of the Increase in Ultraviolet Absorption of the Cytoplasm after X and Gamma Irradiation.

The observed increase in the optical density of the section of tissue which has received X or gamma radiation *in vivo* is of the order of 0·15 at the wave-length 2537 Å for a radiation dose of the order of 500 r, but may be considerably greater. These measurements refer to sections of thickness approximately 2μ; the absolute thickness of the sections is not known with precision, but with all the sections measured the error introduced by the difference in thickness of the sections is negligible in comparison with the observed absorption changes. The photometric measurements of the ultraviolet photomicrographs all refer to the wave-length 2537 Å, so that for their interpretation it is essential to utilize the additional information provided by the measurements of the ultraviolet absorption spectra and the histochemical tests. These experimental findings are consistent with the accumulation in the cytoplasm of proliferating and incompletely differentiated cells of a *pentose* (which is not desoxyribose) and purine (and pyrimidine) derivatives, probably *adenine*, and possibly also guanine in small amounts and uracil. The ultraviolet absorption spectrum of the accumulating substances cannot give any information concerning the pentose groups (cf. Holiday, 1930; Goos, Schlubach, Schröter, 1930). Further, the existence of the long wave-length region of ultraviolet absorption suggests that other chromophoric groups are present, and such groups may of course contribute to the absorption band near 2600 Å. The solubility properties suggest that the accumulating substances are nucleotides or nucleosides.

The increase in cytoplasmic density of 0·15 at the wave-length 2537 Å, with sections 2μ in thickness, can be accounted for by the accumulation of yeast adenylic acid in concentration 2·8 per cent. or phytonucleic acid in concentration 3·5 per cent. These values of course refer to the cytoplasm of the cells of the proliferating and differentiating areas only. The mean concentration for the whole tissue is of much less significance for the present work, because it depends mainly on the proportion of fully differentiated cells present together with the adult connective tissue and blood vessels of the stroma. The macroscopic method of measurement of the absorption spectrum determines the approximate lower limit of the mean concentration for the whole section, and in Case 3, for example, this value is approximately one-tenth of that obtained for the growing areas by the spectroscopic image method.

The possibility that the observed increase in absorption at 2537 Å could be attributed to "photochemical" changes in protein appears to be excluded. The observed changes in the absorption spectrum are quite unlike any of the

known changes produced either by ultraviolet radiation or by very large doses of X or gamma radiation ; further, an increase in density of 0·15 at 2537 Å would require for its explanation an additional concentration or absorption increase of the proteins of the order of 200–700 per cent., and values twice as high as this would be called for to account for some of the experimental results. Quite apart from the improbability of such assumptions, it is evident from the work of Sanigar, Krejci and Kraemer (1939) that no detectable changes would result from photochemical changes produced in the cell proteins *in vitro* by the doses of X and gamma radiations used.

The most striking result of practically all the experiments reported in the literature is the insensitivity of the cell constituents *in vitro* to X and gamma radiations ; almost invariably, detectable chemical changes are found only with necrotic doses (Arnow, 1936 ; Holmes, 1939) ; e.g. Jacobson (1939) could detect no chemical changes in a pterine of physiological importance after 1,000,000 r gamma radiation. However, in a few cases significant effects have been detected with doses within the therapeutic range, e.g. inhibition of glycolysis after irradiation at low temperature (Crabtree and Gray, 1939), inactivation of purified enzymes (Dale, 1940).

In a few instances the number of molecules transformed per ion pair has been measured, e.g. for tyrosine in solution the value is approximately $\phi = \frac{1}{12}$ (Stenström and Lohmann, 1928) ; of particular interest are the results of Gunther and Holzapfel (1939), who found that water vapour and liquid water (but not ice at approximately —180° C.) are decomposed by X radiation with production of 1 H_2 molecule per ion pair (cf. Fricke, 1935).

As a preliminary to the present work a series of experiments (unpublished) have been carried out in collaboration with Dr. C. B. Allsopp on changes in the ultraviolet absorption spectrum of compounds of physiological interest after X and gamma irradiation. Although not completed, these experiments have given maximum values for the number of molecules transformed per ion pair ; the results are for yeast adenylic acid 9, glycyl-tyrosine 11, the tyrosine groups of insulin 27, and indol 18. These are preliminary values, and it appears probable the true values may be much lower in some cases. However, these experiments provided data for calculating the order of the changes to be expected in the histological preparations as a result of purely photochemical changes due to the X and gamma radiation without the intervention of " physiological " processes. In a 2μ layer of a 10 per cent. solution of yeast adenylic acid and for a dose of 1000 r assuming that the number of molecules decomposed per ion pair $\phi = 10$, the optical density would decrease by approximately 10^{-4} ; for a density change of the order of 0·1 (which in this case would be a *decrease* in contrast with the observed increase) ϕ would have to be of the order 10^4. Similarly for a layer 2μ in thickness of a 20 per cent. solution of a protein containing 6 per cent. tyrosine and 2 per cent. tryptophane, and assuming that the chromophor of tryptophane behaves like indol, irradiation with a 1000 r X or gamma radiation would produce a decrease in density of the order of 10^{-5} ; this result is consistent with the experimental findings of Sanigar, Krejci and Kraemer (1939). It can be concluded that even if X and gamma radiations produce photochemical changes with an efficiency as

high as 10 molecules transformed per ion pair, no changes in the ultraviolet absorption of the histological preparations could be detected with a dose of 1000 r. (All the above calculations refer to the wave-length 2537 Å.)

Changes in the optical density of the magnitude observed in the ultraviolet photomicrographic measurements imply that either " photochemical " processes are occurring with extremely high efficiencies of the order 10^3–10^4 molecules transformed per ion pair, or that the absorption changes are due to complex secondary " physiological " processes resulting from photochemical changes occurring with normal efficiencies of the order of unity. Changes of very high efficiency may be explicable in terms of a chain reaction mechanism, and suggest the possibility of enzyme inhibition by the radiation, but the necessary experimental evidence is not yet available.

SUMMARY.

An increase in the absorption of ultraviolet radiation of wave-length 2537 Å in the cytoplasm of proliferating and differentiating cells has been found after therapeutic doses of X and gamma radiations. The dependence of this absorption increase upon dose and dose rate and the influence of recovery processes are discussed. As yet no significant difference in absorption increase has been observed between normal and hyperplastic tissues and malignant tumours.

The increase in ultraviolet absorption of the cytoplasm after irradiation is shown to be due to the accumulation of pentose nucleotides, probably ribonucleotides, containing adenine and some other unidentified chromophoric groups. The magnitude of the change is consistent with the presence in the irradiated cytoplasm of ribonucleotides in local concentration often of the order of 3 per cent., and suggests the production of a metabolic disturbance by the radiation.

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DISTURBANCE OF NUCLEIC ACID METABOLISM PRODUCED BY THERAPEUTIC DOSES OF X AND GAMMA RADIATIONS. PART III: INHIBITION OF SYNTHESIS OF THYMONUCLEIC ACID BY RADIATION.

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Nucleic Acid Content of the Nuclei after Irradiation.

Absolute measurements of the amount of nucleic acid in individual nuclei have been described by Caspersson (1936, 1940). Similar measurements were not possible with the apparatus available, and it has been necessary to employ photographic photometry for measurements of possible changes in absorption occurring in the nuclei after X and gamma irradiation. These measurements give quantitative information concerning the concentration of purine and pyrimidine groups present, but cannot detect changes in the pentose or phosphate groups (cf. Holiday, 1930; Goos, Schlubach and Schröter, 1930). The " nucleic acid " estimated thus includes both ribo- and desoxyribo-nucleic acids and the corresponding nucleotides, nucleosides and bases. Three methods have been employed:

I. *Measurement of mean changes in absorption and size per nucleus.*

Measurements have been made of the density of absorbing materials within the nuclei of the cells of the control and irradiated sections in areas of strictly comparable and carefully selected histological structure. Measurements of the corresponding nuclear diameters were also made in addition to measurements of the absorption of the adjacent cytoplasm.

This method is difficult to apply because of the inhomogeneity of the

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